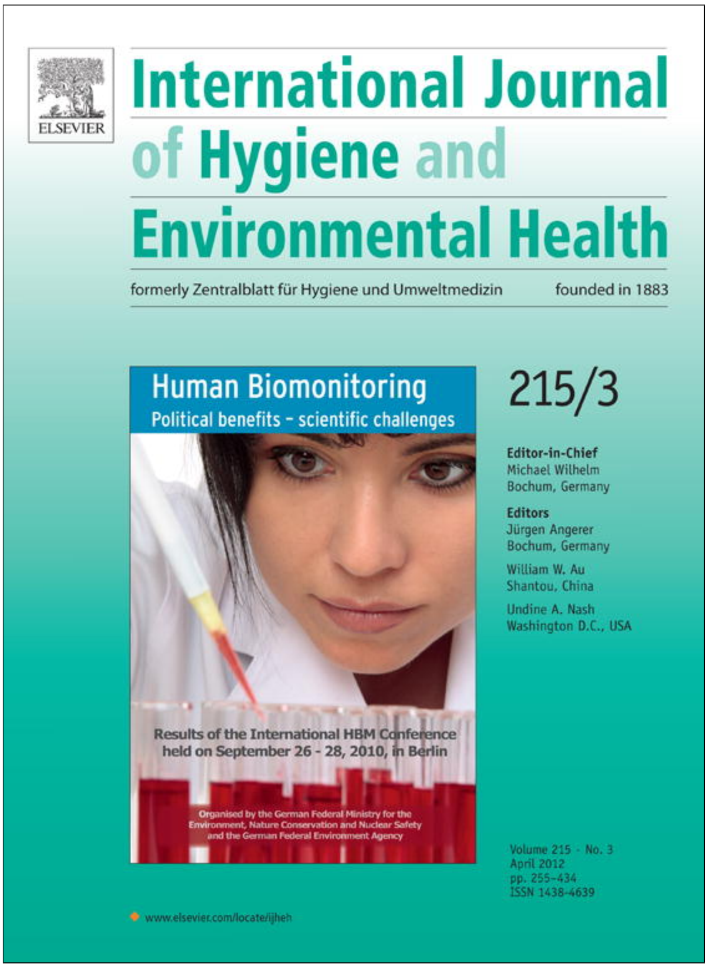
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Determinants of ochratoxin A exposure—A one year follow-up study of urine levels

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ABSTRACT

Dietary exposure to the ochratoxin A (OTA) occurring in Portugal is characterized by a high frequency of contamination of the consumed foodstuffs, although at low levels. The exposure bears significance for the total food consumed, and not for a particular one. Biomonitoring studies are thus fundamental in simplifying the evaluation of exposure, with no need to examine the entire range of consumed foodstuffs. Biomonitoring studies further allow the identification of host factors as predictors of OTA exposure in epidemiologic studies, the results of which are merited for targeting intervention strategies by public health authorities and advising official regulatory decisions. Using a longitudinal approach, this study examined factors related to OTA exposure in the adult population over a one-year period. Anthropometric measures, season of the year and region were the selected factors correlated with OTA exposure biomarker. Urine samples from 95 inhabitants from six Portuguese main geographical areas were assayed through spectrofluorimetric detection. Exposure to OTA proved to markedly increase in winter, and gender differences were observed only in summer, which might be related to different dietary patterns not only between seasons, but also between genders. The same rationale may also serve the observed statistically significant differences between some regions. No other strong association upon the remaining determinants under testing was observed. These observations reinforce the need for OTA exposure evaluation, possibly specifically targeting the staple foods or dietary habits that sustain potential predictors or determinants of exposure.

Introduction

Unlike food additives, mycotoxins occur in unpredictable levels that can vary both temporally between seasons and spatially between different growing areas or under different storage conditions (Shephard, 2008). Their presence in foodstuffs is unavoidable and despite many years of research, coupled with current good practices during food production, storage and distribution, mycotoxins continue to contaminate a wide range of foods and feeds (Giray et al., 2009). First described in the birth of the individualized science of mycotoxicology by Van der Merwe et al. (1965), ochratoxin A (OTA) remains one of the few mycotoxins known to occur in foodstuffs at sufficient levels and frequencies to be merited (Clark and Snedeker, 2006). OTA is a naturally occurring secondary metabolite produced by several toxigenic micromycetes (Ostry et al., 2002) pertaining to the *Aspergillus* and *Penicilium* genera (Logrieco et al., 2003). Together, the specific ecological niches of the ochratoxigenic fungal species cover the entire planet. Considering further the variety of food commodities contaminated (Duarte et al., 2010a; Ghali et al., 2008; Jørgensen, 1998; Tafuri et al., 2004; Vatinno et al., 2008; Visconti et al., 2000; Zimmerli and Dick, 1995; Zinedine et al., 2010) allied to a globalized trade of foods, a worldwide population exposure is expected, as confirmed by the widespread reports on OTA occurrence in all continents (Aksoy et al., 2007; Aoyama et al., 2010; Hocking et al., 2003; Magnoli et al., 2007; Miraglia and Brera, 2002; Ng et al., 2009; Soubra et al., 2009; Wanigasuriya et al., 2008; Zinedine et al., 2010).

OTA concern is further sustained by its toxicological effects, especially nephrotoxicity. Indeed, OTA causes nephropathy in all species tested with large sex and species differences in potency, pigs being most sensitive (Walker and Larsen, 2005). In the 1980s a Porcine Endemic Nephropathy (PEN) was described in Denmark (Jørgensen and Petersen, 2002). With histo-pathological similarities, human nephropathies have also been linked to OTA exposure through epidemiological correlations. That is the case of Balkan Endemic Nephropathy (BEN) in South-eastern European countries (Pfohl-Leszkowicz et al., 2007), and the strikingly resembling Chronic Interstitial Nephropathy (CIN) in the North African

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countries (Grosso et al., 2003), in both of which OTA aetiology has been implicated. However, within the same range of OTA concentrations in blood and/or urine some people develop a disease while others do not, and still comparable levels of toxins have been found in non-endemic countries. This somewhat controversial correlation (Märtlbauer et al., 2009) has a complex nature that may rely on different diets and eating habits, environmental factors and/or genetic predisposition so many times uncared for (Creppy et al., 2005). Furthermore, additive, synergistic or antagonist interaction due to multi-mycotoxin contamination (Stoev, 2010), as well as other risk factors, may also contribute to the complexity of the aetiology assessment. Alongside with the recognized nephrotoxic potential OTA also proved to be hepatotoxic (Chopra et al., 2010), potently teratogenic (Wangikar et al., 2005), immune-modulatory (Müller et al., 2004), and mutagenic (Schilter et al., 2005). Supported by sufficient evidence of carcinogenicity in animal studies, contrarily to inadequate evidence in humans, it is classified as "Possible carcinogenic to humans" (group 2B) in the International Agency for Research on Cancer classification (IARC, 1993) and as "Reasonably anticipated to be carcinogenic to humans" in the US National Toxicology Program (NTP) classification (Abnet, 2007).

The potential toxic effects of the mycotoxin led some organizations to consider prudent to reduce exposure to OTA (Duarte et al., 2010b). As a result, a legal framework governing the maximum levels of OTA in major contributing foodstuffs and beverages was created in the European Union (CEC, 2006, 2010). Accordingly, tolerable intakes were also recommended at a weekly range of 100 (JECFA, 2001) to 120 ng/kg bw (EFSA, 2006). Concerted efforts are thus heading for health surveillance and exposure assessments of these natural contaminants.

For exposure assessments, and taking into account that food is considered to be the primary route of human exposure, the analysis of OTA food content has been the classical epidemiological approach, allowing the identification of major food contributing sources. However, biomonitoring stands as a major alternative, allowing from the analysis of a single biological sample to evaluate exposure derived from all routes and all sources and bioavailability (Duarte et al., 2011), and further avoiding the heterogeneous nature of mycotoxin food contamination (Shephard, 2008). Biomonitoring of OTA has thus become a gold standard in the evaluation of exposure. The use of biomarkers of exposure is further favored by the long half-life of the mycotoxin in the body, resulting from an extensive reabsorption along the nephron (Blank and Wolffram, 2005), affinities of the toxin for plasma proteins as well the possibility of enterohepatic recirculation that additionally retain OTA in the circulation (Stander et al., 2001). Despite resulting in relatively higher OTA blood levels, thus requiring less sensitive methods, the long half-life of the toxin also contributes to a steady-state concentration of OTA in blood samples from humans with a frequent dietary exposure (EFSA, 2006). Other shortcomings of blood matrices are related to the necessarily invasive collection, need of medical personnel involvement, and short-time indication. Urinary OTA exposure biomarker is a non-invasive alternative, increasingly important with the advent of more sensible and accurate methodologies of analysis which mitigates the low levels at which it occurs. Contrarily to blood-based biomarkers compromised by the steady-state concentration, OTA in urine proved to correlate more closely with the level of consumption among the general population (Gilbert et al., 2001). In addition, it circumvents the need of technicians for collection, adding ease and fastness to the collection procedure, thus enabling a comparatively more keen involvement of participants as compared to blood (Duarte et al., 2011). Thus in recent years, increasing reports of small scale populations surveys have been made, whether in healthy inhabitants of non-endemic (Breitholtz-Emanuelsson et al., 1994; Gilbert et al., 2001; Duarte et al., 2009; Akdemir et al., 2010; Rubert et al., 2011) or endemic regions for suspected OTA-related nephropathies (Domijan et al., 2009; Petkova-Bocharova et al., 2003). Not often have OTA urine levels been used in nationwide scale surveys, like in Hungary (Fazekas et al., 2005), Bulgaria (Nikolov et al., 2002), and Portugal (Duarte et al., 2010c). All the published data is derived from one point of collection, with the exception of the studies of Gilbert et al. (2001) and Castegnaro et al. (2006) in which the studied population was followed during a one-month period to test relations with the ingested food. Still, in both no relations were sought concerning individual characteristics, and little to no information is available on the medium-term OTA urine levels and seasonal variations for individual people.

Fig. 1. Map of the surveyed Portuguese regions (shadowed grey).

Alternative biomarkers of exposure in urine include OTA metabolites and/or conjugates such as ochratoxin alpha (OTα) (Muñoz et al., 2010; Coronel et al., 2011). Although it is true that because OTA occurs at quite low levels in urine, the analysis of OTα may increase sensitivity and facilitate biomonitoring, the reported substantial interindividual variation on human ability for OTA detoxification might greatly compromise the use of OTα as an alternative or simply additional biomarker.

In this context the herein reported study was aimed to follow for one year the occurrence of OTA in a cohort of healthy adult individuals and to investigate the extent to which the urinary levels were constant between seasons and influenced by individual characteristics.

Materials and methods

Study cohort and sampling criteria

The studied population consisted of 95 inhabitants of six regions of the Portuguese mainland (*vide* Table 1), specifically Bragança, Porto, Coimbra, Lisbon, Alentejo, and Algarve (Fig. 1), in an attempt to representatively screen and follow-up the Portuguese inhabitants during one year.

A total of 50 women and 45 men were enrolled (female ratio 1.1). Anthropometric measurements were taken at baseline, along with a small questionnaire that provided information regarding region, gender and age. Anthropometric information recorded at baseline is described in Table 2 according to gender. Body mass index (BMI) was calculated as defined by the World Health Organization – WHO

Table 1
Regional distribution of the participants.

Region	Number	Gender		Age (years)		Height (cm)		Weight (kg)	
		Female	Male	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Bragança	18	9	9	36.8 ± 11.6	[20;56]	169.4 ± 11.2	[150;192]	69.2 ± 15.7	[48;115]
Porto	19	10	9	53.7 ± 19.0	[24;83]	169.2 ± 8.2	[155;183]	66.9 ± 11.5	[47;90]
Coimbra	6	3	3	42.0 ± 14.0	[23;56]	171.0 ± 14.0	[150;188]	70.0 ± 17.1	[51;98]
Lisbon	35	17	18	45.7 ± 14.0	[23;69]	168.1 ± 8.9	[149;185]	72.7 ± 11.8	[53;108]
Alentejo	5	3	2	50.0 ± 20.6	[23;81]	162.4 ± 7.6	[152;173]	67.6 ± 8.0	[57;77]
Algarve	12	8	4	43.8 ± 13.2	[26;71]	164.7 ± 7.3	[157;178]	73.9 ± 19.1	[49;120]
Total	95	50	45	45.4 ± 15.6	[20;83]	168 ± 9.3	[149;192]	70.6 ± 13.7	[47;120]

(WHO, 1995), i.e. the weight in kilograms divided by the square of the height in meters (kg/m^2).

The enrolled subjects were selected by a simple random process. All respected the criteria of being healthy, with no history of hepatic or nephropatic conditions.

The individuals were divided in different categories regarding region (Bragança, Porto, Coimbra, Lisboa, Alentejo, Algarve) and gender (female, male), and cut-off points regarding age ([20;29], [30;39], [40;49], [50;59], [60;69], [70;79], [80;89] years), height (<160, [160;169], [170;179], [180;189], >190 cm), weight (<50, [50;59], [60;69], [70;79], [80;89], >90 kg) and BMI (<18.5, [18.5;24.9], [25;29.9], [30;34.9], [35;39.9], >40) were established.

Sample collection and storage

The recruitment occurred in 2007, and the two selected collection periods were the winter of 2007 and the summer of 2008. The 95 volunteers participated in both collection periods and were given clean 50 ml non-sterile plastic vessels with screw cap and were requested to fill it up with first morning midstream urine the day they were instructed to. The filled plastic vessels with the fasting urine sample were returned to the laboratory in cold chain by icebox, and kept at −20 °C until extraction.

Ethic considerations

The participants were all adults and signed a written informed consent form. The study was performed in accordance to the Declaration of Helsinki.

Analytical methods

The applied methodology for OTA urine analysis was previously validated (Pena et al., 2006) and applied (Duarte et al., 2009, 2010c). In brief, it included extraction of 10 ml of the sample with an equal volume of 5% $NaHCO_3$, filtration and clean-up through immunoaffinity columns (Ochratest, Vicam^{TM}). After washing the column with MilliQ water, OTA was eluted with 3 ml methanol. The methanol was evaporated under a gentle nitrogen flow in a water bath at 40 °C. The dried extract was stored at −20 °C until immediately before injection, when it was dissolved in 125 μl of mobile phase. High-performance liquid chromatography coupled with fluorescence detection (HPLC–FD) analysis was performed in a Perkin-Elmer Model LS45 spectrofluorimeter (Beaconsfield, UK) using a vacuum-filtered solution of acetonitrile/water/acetic acid (49.5:49.5:1.0, v/v/v) as mobile phase, flowing at a 1 ml/min rate. Wavelengths in the spectrofluorimeter were set at 333 nm for excitation and 460 nm for emission, both with a spectral bandwidth of 10 nm. For quantification purposes, a 0.01 μg/ml OTA working solution was injected between each sample. All injections were of a volume of 20 μl.

The OTA peak identity confirmation was done by conversion of OTA into its methyl ester form (Castegnaro et al., 1990; Duarte et al., 2010c) followed by analysis under the HPLC–FD conditions applied in real samples.

Revalidation assays were performed by spiking triplicate OTA-free urine samples, during three days, at spiking levels of 0.02, 0.05 and 0.1 ng/ml. After fortification, the sample was left to stand in the dark for 15 min, after which the described protocol was followed. Linearity for OTA quantification was verified through a calibration curve obtained by using the linear least squares regression procedure of the peak area *versus* the concentration of working standard solutions at three determinations of four concentration levels (1.0, 2.0, 5.0, and 10.0 ng/ml).

Statistical analysis

Possible significance of correlation between the measured parameters and OTA urine levels was examined by simple and multiple regression analysis. For statistical analysis, when the concentration was below the limit of quantification (LOQ) it was set to 50% of that limit.

The nonparametric statistics used in this work, sign tests, median tests, Mann–Whitney, Spearman's rho and the Wilcoxon matched pairs (signed ranks) tests, were carried out following classical methods, as implemented in the statistical software Statistica for Windows (StatSoft – STATISTICA, data analysis software system/version 7).

Results

Analytical performance

Recoveries ranged from 96.4 to 100.5%, intraday relative standard deviation (RSD) from 7.39 to 9.30%, and interday RSD from 4.12 to 12.93%. The calibration curve in the linearity assay showed a coefficient of correlation of 0.9998.

The limit of quantification (LOQ), determined as the lowest OTA concentration at which a sample could be spiked and still originate accurate and repeatable results with the application of the

Table 2
Baseline anthropometric characteristics of the participants according to gender.

Gender	Number	Age (years)		Height (cm)		Weight (kg)		BMI (kg/m^2)	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Female	50	46.1 ± 14.3	[20;83]	161.8 ± 6.5	[149;175]	63.9 ± 10.1	[47;89]	24.4 ± 3.8	[17.6;33.6]
Male	45	44.5 ± 17.1	[22;81]	174.9 ± 6.9	[161;192]	78.1 ± 13.4	[55;120]	25.5 ± 4.1	[18;41]
Total	95	45.4 ± 15.6	[20;83]	168.0 ± 9.3	[149;192]	70.6 ± 13.7	[47;120]	24.9 ± 3.96	[17.6;41]

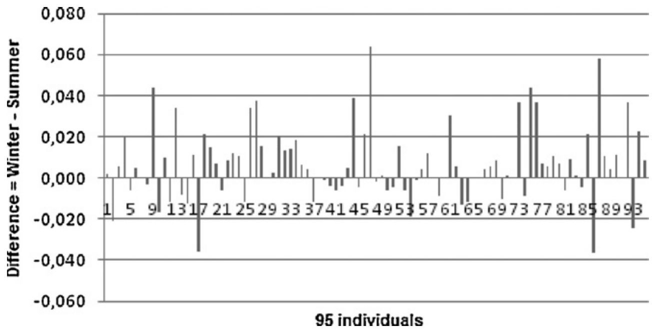

Fig. 2. Distribution of differences in OTA levels between winter and summer in each study subject.

experimental procedure (signal-to-noise ratio of approximately 10:1), was set at 0.008 ng/ml. Limit of detection (LOD), determined as the lowest concentration detected (signal-to-noise ratio of approximately 3:1), was set at 0.0024 ng/ml.

Ota urine levels

The testing results in the two collection periods are summarized in Table 3 according to region and selected individual characteristics. Overall, winter and summer samples of the 95 participants featured an incidence of 87.4% and 81.1% and an average level of 0.022 ± 0.015 and 0.016 ± 0.008 ng/ml, respectively. The highest value was 0.071 ng/ml.

Statistical analysis

It is important to emphasize that a normal distribution of OTA content in urine samples was not observed, as illustrated in the graph drawn by considering for each individual the differences in OTA levels between winter and summer (*vide* Fig. 2). This observation was supported by a Kolmogorov–Smirnof test ($d = 0.118$, $p < 0.15$) and a Shapiro–Wilk's test ($W = 949$, $p = 0.00096$). Furthermore, differences tended to be positive (Fig. 2), indicating that OTA levels were higher in winter than in summer. These findings were in agreement with the higher kurtosis and skewness values observed in winter in both genders (data not shown).

Separating data by gender and drawing histograms (not shown), it was possible to observe higher OTA levels in winter than in summer, and differences between genders seemed to be present. In order to clarify these observations, Mann–Whitney *U* tests were carried out comparing OTA levels in females and males in each season. According to such tests the observed differences between females and males in winter were not significant ($p = 0.24$), contrarily to the same comparison in summer ($p = 0.02$), with males showing lower OTA levels. On the basis of non-parametric descriptive statistics (quartiles and extreme values as summarized in Fig. 3) it become evident that main differences were related to a few individuals displaying much higher levels than the majority, which was observed mainly in winter. Furthermore, as clearly shown in Fig. 3, the majority of men (as a group) presented lower OTA levels in summer.

It was deemed important to complement these general conclusions with a study based on the results obtained for each individual, following the graph depicted in Fig. 2. To achieve such an analysis, Sign test and Wilcoxon matched-pairs test (Wilcoxon signed ranks test) were used. The former evaluated the number of individuals for whom an increase or decrease in OTA levels between winter and summer was observed, while the latter carried out the same comparison, but taking into consideration the absolute value of the variations observed *per* individual. Both tests led to the same conclusion: a highly significant decrease in OTA levels from winter to summer was observed for men (Sign test $p = 0.0034$; Wilcoxon matched-pairs test $p = 0.0007$), with no significant differences observed for women (Sign test $p = 0.4610$; Wilcoxon matched-pairs test $p = 0.3616$). Spearman's rho was also calculated for each gender, according to which there are no significant correlations in the OTA levels featured by both groups (rho ≈ −0.06 for women and rho ≈ −0.04 for man).

The analysis of differences among regions was carried out following the Kruskal–Wallis ANOVA by Ranks. Differences among regions existed in both seasons ($p = 0.0487$ for winter and $p = 0.0048$ for summer), being higher in summer. Differences between regions were compared in pairs, with the only significant difference being found between Bragança and Algarve in summer ($p = 0.0459$). Box-and-Whisker's plots for all regions per season are shown in Fig. 4. It becomes evident that, although some of the differences observed among regions were not statistically significant, probably larger samples would lead to an increase in the significance of the differences observed.

Regarding age, there was no correlation with the absolute values of OTA (in winter $p = 0.06$; in summer $p = -0.09$) or with the differences observed between winter and summer ($p = 0.09$). Furthermore, and as stated above, there was no variation inside each age group attributable to gender, as there was not variation inside each gender attributable to age grouping because no significant interactions were found between the two factors.

As for height and weight, a variate analysis of these two variation factors demonstrated no significant differences for neither women ($p = 0.2662$ and $p = 0.4369$) nor men ($p = 0.1127$ and $p = 0.1181$), respectively. The same analysis, but applied to the BMI categories against the differences in the OTA levels between both collection periods showed significant differences ($p = 0.0131$) but only because of missing data vitiated. In fact, by removing category [35–39.9] – with no observation, and [>40] – with only one observation, the results turn to non-significant ($p = 0.1443$). Thus, considering this population, variations in OTA urine content were independent of age, height, weight and BMI.

Discussion

This study enrolling healthy individuals provided the opportunity to follow-up the effect of season on the levels of urinary OTA, and search for possible correlations with basic anthropometric measures and region.

The first challenge of this biomonitoring study was participant interest in taking part with the recruitment having been further constrained by institutional rules, privacy issues and individual reluctance of each subject, making it labor-intensive. This justifies the fact that of the initial group, only 95 subjects persisted through the two collection periods. Furthermore, it can also explain the lack of studies of such nature related to mycotoxins. To our knowledge this is the first OTA follow-up study performed in urine samples. In general, epidemiological population-based studies on OTA exposure have mostly been focused on blood levels.

The obtained results clearly show a high within-subject variation of OTA urine content. Therefore, it is reasonable to consider that OTA urine levels, like OTA serum levels (Palli et al., 1999), have a limited use at the individual level but can be used to characterize populations or subgroups of subjects. The high within-subject variability is also highly suggestive and consistent with OTA in urine being a short-time biomarker.

It is possible in this way to identify the strongest determinants of OTA urine levels, considering the group of enrolled participants. According to the statistical analysis of the obtained results, the main determinants among the ones considered in this study were season

Table 3
Frequency, mean and range values of OTA urine levels (ng/ml) according to the selected characteristics in the two collection periods (winter and summer).

Characteristics	*n*	Frequency of detection		Mean ± SD[a]		Range	
		Winter	Summer	Winter	Summer	Winter	Summer
Region							
Bragança	18	100%	72.2%	0.021 ± 0.010	0.013 ± 0.006	<LOQ–0.042	n.d.–0.022
Porto	19	94.7%	57.9%	0.021 ± 0.014	0.017 ± 0.011	n.d.–0.062	n.d.–0.040
Coimbra	6	83.3%	100%	0.010 ± 0.001	0.013 ± 0.006	n.d.–0.011	<LOQ–0.022
Lisboa	35	71.4%	88.6%	0.025 ± 0.019	0.013 ± 0.006	n.d.–0.071	n.d.–0.033
Alentejo	5	100%	100%	0.022 ± 0.012	0.019 ± 0.004	<LOQ–0.039	0.014–0.025
Algarve	12	100%	91.7%	0.022 ± 0.017	0.023 ± 0.009	<LOQ–0.068	n.d.–0.039
Gender							
Female	50	86.0%	84.0%	0.021 ± 0.015	0.016 ± 0.007	n.d.–0.062	n.d.–0.040
Male	45	88.9%	77.8%	0.023 ± 0.016	0.016 ± 0.010	n.d.–0.071	n.d.–0.039
Age (years)							
[20;29]	16	75.0%	75.0%	0.021 ± 0.011	0.020 ± 0.013	n.d.–0.039	n.d.–0.040
[30;39]	21	71.4%	95.2%	0.026 ± 0.016	0.016 ± 0.007	n.d.–0.068	n.d.–0.033
[40;49]	25	96.0%	80.0%	0.018 ± 0.012	0.017 ± 0.006	n.d.–0.062	n.d.–0.028
[50;59]	17	100%	76.5%	0.023 ± 0.017	0.014 ± 0.005	>LOQ–0.054	n.d.–0.025
[60;69]	8	87.5%	87.5%	0.030 ± 0.031	0.011 ± 0.003	n.d.–0.071	n.d.–0.014
[70;79]	4	100%	50.0%	0.016 ± 0.007	0.023 ± 0.016	0.008–0.023	n.d.–0.035
[80;89]	4	100%	75.0%	0.022 ± 0.004	0.011 ± 0.004	<LOQ–0.027	n.d.–0.016
Height (cm)							
<160	20	90.0%	85.0%	0.020 ± 0.013	0.013 ± 0.004	n.d.–0.055	n.d.–0.022
[160;169]	32	84.4%	81.3%	0.022 ± 0.017	0.017 ± 0.008	n.d.–0.062	n.d.–0.040
[170;179]	30	90.0%	76.7%	0.025 ± 0.016	0.019 ± 0.010	n.d.–0.071	n.d.–0.039
[180;189]	12	83.3%	83.3%	0.018 ± 0.010	0.014 ± 0.008	n.d.–0.038	n.d.–0.028
>190	1	100%	100%	0.021	0.009	n.a.	n.a.
Weight (kg)							
<50	3	100%	66.7%	0.024 ± 0.015	0.011 ± 0.002	0.010–0.041	n.d.–0.012
[50;59]	17	76.5%	76.5%	0.020 ± 0.015	0.018 ± 0.009	n.d.–0.062	n.d.–0.040
[60;69]	25	84.0%	84.0%	0.017 ± 0.012	0.015 ± 0.005	n.d.–0.055	n.d.–0.027
[70;79]	29	93.1%	79.3%	0.025 ± 0.016	0.017 ± 0.009	n.d.–0.071	n.d.–0.039
[80;89]	14	85.7%	85.7%	0.025 ± 0.016	0.014 ± 0.005	n.d.–0.054	n.d.–0.020
>90	7	100%	85.7%	0.026 ± 0.019	0.023 ± 0.013	0.008–0.068	n.d.–0.035
BMI (kg/m^2)							
<18.5	3	100%	66.7%	0.025 ± 0.015	0.015 ± 0.005	0.010–0.041	n.d.–0.018
[18.5;24.9]	49	79.6%	79.6%	0.021 ± 0.013	0.017 ± 0.009	n.d.–0.062	n.d.–0.040
[25;29.9]	32	93.8%	78.1%	0.023 ± 0.018	0.015 ± 0.006	n.d.–0.071	n.d.–0.027
[30;34.9]	10	100%	100%	0.022 ± 0.016	0.013 ± 0.005	<LOQ–0.054	<LOQ–0.020
[35;39.9]	–	–	–	–	–	–	–
>40	1	100%	100%	0.023	0.035	n.a.	n.a.

[a] Above the LOQ values only; LOD – limit of detection; n.d. – non detected; n.a. – non-applicable; <LOQ – positive but below the LOQ.

of urine collection (winter *versus* summer) and gender (man *versus* woman), in a multivariate approach, in addition to region.

Regarding seasonal variation overall, a trend towards higher OTA winter values in contrast to summer ones is obvious, but it must be emphasized that the magnitude of such difference is highly variable between individuals. Indeed, particular climatic conditions could have been responsible for a higher contamination of the ingested food in the period preceding collection, as well as a possible seasonal variation of the dietary and drinking habits that could lead to a different intake during different periods of the year. Evaluation of average and specific short-term individual dietary information would be necessary to clarify this issue (Palli et al., 1999). No study of OTA in urine is available for comparison and interpretation of the seasonal variation. However, most studies of OTA in blood do show opposite results, i.e. increase of summer OTA levels. For instance, higher values were reported in blood samples collected during the summer in several Mediterranean countries, like Italy (Palli et al., 1999), Turkey (Erkekoğlu

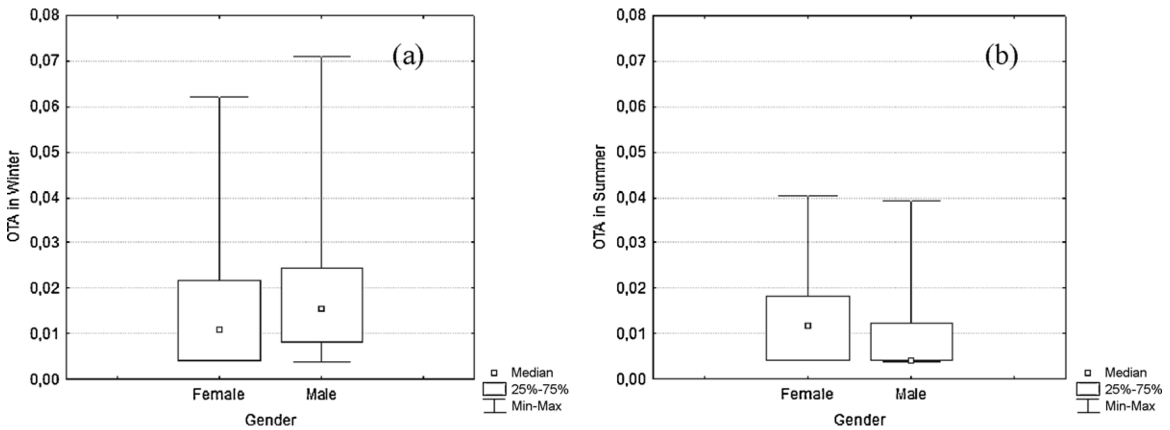

Fig. 3. OTA levels in winter (a) and summer (b), for males and females, displayed as Box-and-Whisker's plots based on medians, quartiles and extreme values.

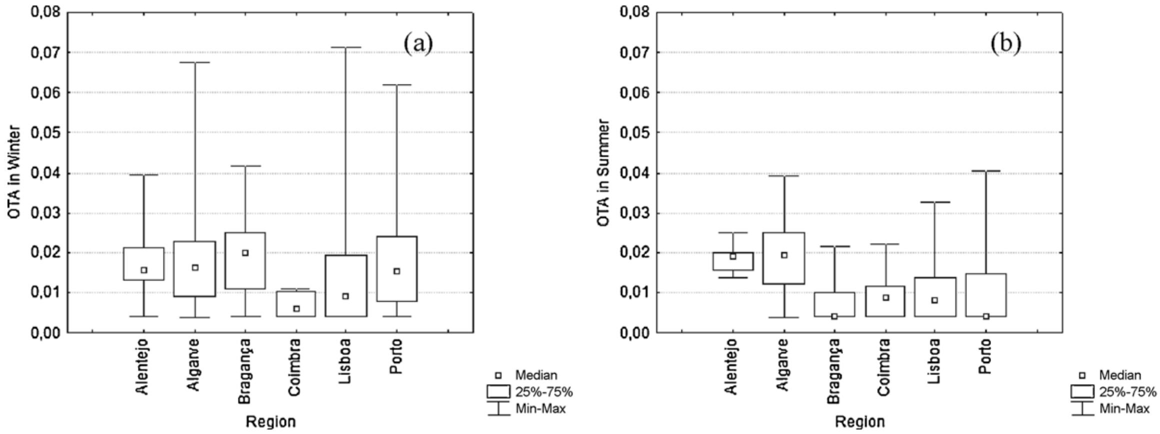

Fig. 4. Variation in OTA levels per region in winter (a) and summer (b), displayed as Box-and-Whisker's plots based on medians, quartiles and extreme values.

et al., 2010), and Croatia (Domijan et al., 1999; Peraica et al., 2001). In urine of children (<5 years old) from Sierra Leone, Jonsyn-Ellis (2000) observed a higher incidence of OTA contamination during the collection period of dry season comparatively to the rainy season (correspondingly 14.5% and 10.8%).

Regarding the gender factor, the differences were only observed in summer, with males showing lower OTA levels. Already before, during conventional biomonitoring surveys of Portuguese inhabitants during winter, no significant differences were found between male- and female-provided samples, neither on a nationwide-scale (Duarte et al., 2010c) nor at a more narrow-scale (Duarte et al., 2009; Manique et al., 2008; Pena et al., 2006). The only exception was the 20–39 years group of the study of Pena et al. (2006), in which male-provided samples presented higher values of incidence and average level. In view of these earlier results, allied with the absence of significant differences reported by surveys in foreign populations (Fazekas et al., 2005; Coronel et al., 2011), little to no difference between genders was expected. But on the other hand, one could anticipate gender effect as a key determinant with a role in OTA metabolism or simply reflecting different exposure levels between genders, ultimately attributable to different dietary patterns or quantity of ingested food. Actually, renal structure and functions under various physiological, pharmacological, and toxicological conditions were already shown to be different between genders, which may reflect gender-hormone-regulated expression and action of transporters in the apical and basolateral membranes of nephron epithelial cells; specifically, renoprotective effects of estrogens were demonstrated in nephrotoxicity induced by OTA (Sabolić et al., 2007). Nevertheless, it is important to underline that such weight of evidence does not explain the difference obtained between OTA urine concentrations between genders; it merely suggests that further work is missing, for instance regarding the susceptibility of each gender in nephropathic condition presumably induced by or in which OTA might be involved, like BEN and/or CIN (Duarte et al., 2011).

Additionally, exposure levels could also reflect different consumption patterns or simply amount of ingested food potentially contaminated. In fact, as demonstrated for the European population (Miraglia and Brera, 2002), wine, beer and coffee are, following cereals and their derivatives, the major contributors to OTA exposure. Their consumption is definitely higher for the male gender which could also induce a potential gender-related difference. The recognized low level contamination of a wide range of different consumed foods may however attenuate such contribution (Duarte et al., 2010d), and thus dilute potential gender-related differences of consumption.

A further selected determinant of OTA urine levels, height was thought to be a determinant, mirroring possible gender differences, since the taller individuals are usually men. Nevertheless, no statistical significance was observed, just as for other selected individual characteristics. Ultimately these findings suggest that OTA contamination is widespread in foods consumed by the Portuguese population, regardless of age, height and weight. In other words, the number of different contaminated foods contributing to this exposure is probably broaden and transversal to the entire population. This is in fact in agreement with previous reports from Portugal and other countries, in which a wide range of foods has been identified as being contaminated. In a recent review (Duarte et al., 2010d), of all the commodities for which the contamination levels have been ascertained in Portugal through individual studies, bread was the major contributing food, fitting in the "transversal" food to all ages, regardless the gender, region or economic power. However additional analyses are needed to explore the dietary determinants of OTA levels in this population (Palli et al., 1999). Significant correlations between OTA urine contamination levels and age were also absent in previous studies, whether considering average daily OTA excretion (Akdemir et al. (2010), or conventional point observations with morning urine (Coronel et al., 2011)).

Concerning region, a significant difference was only observed between Bragança and Algarve regions, once again greater in the summer. Without data from food intake (types of foods, consumption and corresponding contamination level) one can only speculate on the rationales of such difference. Bragança is the Northern- and innermost region and Algarve the most southern of the regions studied (Fig. 1). The Portuguese mainland presents a typical Mediterranean climate, although the influence of factors such as the Atlantic Ocean and the landscape results in obvious contrasts, by provoking a degradation of the typically Mediterranean characteristics. So the Mediterranean climate loses its characteristics on the mainland from South to North and from the coast to the interior. A Mediterranean climate is characterized by having a long, hot, and dry summer period, and a moderate winter, with a relatively low total atmospheric precipitation, while the Atlantic influence increases the overall humidity (Duarte et al., 2010d; IGP, 2010; Minerva, 2010). Climatic conditions conductive to mold spoilage, especially during storage, can thus be echoed. Though significant in view of the specificity of the ecological niches of each ochratoxigenic species, regional climate conditions are not the only influential factor. Between regions different dietary habits persist, explained by different food consumption patterns or socio-economical power. Bragança is the region that presents lower human, social and economical development rates from all the six regions considered, while Algarve has one of the highest

(DPP, 2002). Geographical variations observed can also derive from differences in the ingestion of contaminated foodstuffs, namely in respect to dietary habits or origin of the food consumed (Duarte et al., 2011). Innermost peripheral regions are more dependent on locally grown or stored foodstuffs that can influence mold growth and mycotoxin production in a different way than in the central or littoral regions for the existing variations of climate and humidity levels. Bigger population centers are more dependent on imported supplies. In Algarve, a famous beach destination region, tourist movement during summer results in a striking (temporary) population increase. Regional differences have also been observed in OTA exposure evaluation using blood biomarkers in several countries, like Lebanon (Assaf et al., 2004), Argentina (Pacin et al., 2008), and Tunisia (Grosso et al., 2003; Maaroufi et al., 1995).

Conclusion

Understanding the relationships between general host factors and the variability in urine OTA levels may help in identifying susceptible populations and for targeting specific public health interventions for the prevention of OTA exposure. In this limited longitudinal study, season, gender, and region were the major determinants of the amount of OTA in urine. The low levels of OTA in urine, a common feature of this exposure biomarker, are an obvious disadvantage when looking for determinants of such exposure, limiting statistical analysis. Thus our findings should be further explored in subsequent studies to clarify interpretation of these observations.

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References

Abnet, C.C., 2007. Carcinogenic food contaminants. Cancer Invest. 25, 189–196.
Akdemir, C., Ulker, O.C., Basaran, A., Ozkaya, S., Karakaya, A., 2010. Estimation of ochratoxin A in some Turkish populations: an analysis in urine as a simple, sensitive and reliable biomarker. Food Chem. Toxicol. 48, 877–882.
Aksoy, U., Eltem, R., Meyvaci, K.B., Altindisli, A., Karabat, S., 2007. Five-year survey of ochratoxin A in processed sultanas from Turkey. Food Addit. Contam. 24, 292–296.
Aoyama, K., Nakajima, M., Tabata, S., Ishikuro, E., Tanaka, T., Norizuki, H., Itoh, Y., Fujita, K., Kai, S., Tsutsumi, T., Takahashi, M., Tanaka, H., Iizuka, S., Ogiso, M., Maeda, M., Yamaguchi, S., Sugiyama, K., Sugita-Konishi, Y., Kumagai, S., 2010. Four-year surveillance for ochratoxin a and fumonisins in retail foods in Japan. J. Food Protect. 73, 344–352.
Assaf, H., Betbeder, A.-M., Creppy, E.E., Pallardy, M., Azouri, H., 2004. Ochratoxin A levels in human plasma and foods in Lebanon. Hum. Exp. Toxicol. 23, 495–501.
Blank, R., Wolffram, S., 2005. Effect of dietary sodium bicarbonate supplementation on the toxicokinetics of ochratoxin A in pigs. Mycotoxicol. Res. 21, 147–149.
Breitholtz-Emanuelsson, A., Minervini, F., Hult, K., Visconti, A., 1994. Ochratoxin A in human serum samples collected in southern Italy from healthy individuals and individuals suffering from different kidney disorders. Nat. Toxins 2, 366–370.
Castegnaro, M., Canadas, D., Vrabcheva, T., Petkova-Bocharova, T., Chernozemsky, I.N., Pfohl-Leszkowicz, A., 2006. Balkan endemic nephropathy: role of ochratoxins A through biomarkers. Mol. Nutr. Food Res. 50, 519–529.
Castegnaro, M., Maru, V., Maru, G., Ruiz-Lopez, M.D., 1990. High performance liquid chromatography determination of ochratoxin A and its 4R-4-hydroxy metabolite in human urine. Analyst 115, 129–131.
CEC—Commission of the European Communities, 2006. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Union L364, 5–24.
CEC—Commission of the European Communities, 2010. Commission Regulation (EC) No 105/2010 of 5 February 2010 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards Ochratoxin A. Off. J. Eur. Union L35, 7–8.
Chopra, M., Link, P., Michels, C., Schrenk, D., 2010. Characterization of ochratoxin A-induced apoptosis in primary rat hepatocytes. Cell Biol. Toxicol. 26, 239–254.
Clark, H.A., Snedeker, S.M., 2006. Ochratoxin a: its cancer risk and potential for exposure. J. Toxicol. Environ. Health B 9, 265–296.
Coronel, M.B., Marin, S., Tarragó, M., Cano-Sancho, G., Ramos, A.J., Sanchis, V., 2011. Ochratoxin A and its metabolite ochratoxin alpha in urine and assessment of the exposure of inhabitants of Lleida, Spain. Food Chem. Toxicol. 49, 1436–1442.
Creppy, E.E., Moukha, S., Bacha, H., Carratu, M.R., 2005. How much should we involve genetic and environmental factors in the risk assessment of mycotoxins in humans? Int. J. Environ. Res. Public Health 2, 186–193.
Domijan, A.M., Peraica, M., Fuchs, R., Lucić, A., Radić, B., Balija, M., Bosanac, I., Grgičević, D., 1999. Ochratoxin A in blood of healthy population in Zagreb. Arh. Hig. Rada Toksikol. 50, 263–271.
Domijan, A.-M., Peraica, M., Markov, K., Fuchs, R., 2009. Urine ochratoxin A and sphinganine/sphingosine ratio in residents of the endemic nephropathy area in Croatia. Arh. Hig. Rada Toksikol. 60, 387–393.
DPP—Departamento de Prospectiva e Planeamento, 2002. População e Desenvolvimento Humano: Uma Perspectiva de Quantificação 1970–1999. Ministério do Planeamento, Lisboa.
Duarte, S.C., Bento, J.M.V., Pena, A., Lino, C.M., 2009. Ochratoxin A exposure assessment of the inhabitants of Lisbon during winter 2007/2008 through bread and urine analysis. Food Addit. Contam.—Part A 10, 1411–1420.
Duarte, S.C., Bento, J., Pena, A., Lino, C.M., Delerue-Matos, C., Oliveira, M.B.P.P., Alves, M.R., Pereira, J.A., 2010a. Influencing factors on bread-derived exposure to ochratoxin A: type, origin and composition. Food Chem. Toxicol. 48, 2139–2147.
Duarte, S.C., Lino, C.M., Pena, A., 2010b. Mycotoxin food and feed regulation and the specific case of ochratoxin A: a review of the worldwide status. Food Addit. Contam.—Part A 27, 1440–1450.
Duarte, S., Bento, J., Pena, A., Lino, C.M., Delerue-Matos, C., Oliva-Teles, T., Morais, S., Correia, M., Oliveira, M.B.P.P., Alves, M.R., Pereira, J.A., 2010c. Monitoring of ochratoxin A exposure of the Portuguese population through a nationwide urine survey—Winter 2007. Sci. Tot. Environ. 408, 1195–1198.
Duarte, S.C., Pena, A., Lino, C.M., 2010d. Ochratoxin A in Portugal: a review to assess human exposure. Toxins 2, 1225–1249.
Duarte, S.C., Pena, A., Lino, C.M., 2011. Human ochratoxin A biomarkers—from exposure to effect. Crit. Rev. Toxicol. 41, 187–212.
EFSA—European Food Safety Authority, 2006. Question no EFSA. Q-2005-154. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to ochratoxin A in food. EFSA J. 365, 1–56.
Erkekoğlu, P., Sabuncuoğlu, S., Aydın, S., Şahin, G., Giray, B., 2010. Determination of seasonal variations in serum ochratoxin A levels in healthy population living in some regions of Turkey by enzyme-linked immunosorbent assay. Toxicon 55, 507–513.
Fazekas, B., Tar, A., Kovacs, M., 2005. Ochratoxin a content of urine samples of healthy humans in Hungary. Acta Vet. Hung. 53, 35–44.
Ghali, R., Hmaissia-khlifa, K., Ghorbel, H., Maaroufi, K., Hedili, A., 2008. Incidence of aflatoxins, ochratoxin A and zearalenone in tunisian foods. Food Control 19, 921–924.
Gilbert, J., Brereton, P., MacDonald, S., 2001. Assessment of dietary exposure to ochratoxin A in the UK using a duplicate diet approach and analysis of urine and plasma samples. Food Addit. Contam. 18, 1088–1093.
Giray, B., Atasayar, S., Sahin, G., 2009. Determination of ochratoxin A and total aflatoxin levels in corn samples from Turkey by enzyme-linked immunosorbent assay. Mycotoxicol. Res. 25, 113–116.
Grosso, F., Saïd, S., Mabrouk, I., Fremy, J.M., Castegnaro, M., Jemmali, M., Dragacci, S., 2003. New data on the occurrence of ochratoxin A in human sera from patients affected or not by renal diseases in Tunisia. Food Chem. Toxicol. 41, 1133–1140.
Hocking, A.D., Varelis, P., Pitt, J.I., Cameron, S., Leong, S., 2003. Occurrence of ochratoxin A in Australian wine. Aust. J. Grape Wine Res. 9, 72–78.
IARC—International Agency for Research on Cancer, 1993. Some Naturally Occurring Substances, Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins [Monograph no. 56]. IARC, Lyon.
IGP—Instituto Geográfico Português, 2010. Atlas de Portugal—Um país de área repartida, Available at: http://www.igeo.pt/atlas/Cap1/Cap1.html.
JECFA—Joint FAO/WHO Expert Committee on Food Additives, 2001. Safety Evaluation of Certain Mycotoxins in Food. WHO Food Additives Series 47; FAO Food and Nutrition Paper 74, Available at: http://www.inchem.org/documents/jecfa/jecmono/v47je01.htm.
Jonsyn-Ellis, F.E., 2000. Seasonal variation in exposure frequency and concentration levels of aflatoxins and ochratoxins in urine samples of boys and girls. Mycopathologia 152, 35–40.
Jørgensen, K., Petersen, A., 2002. Content of ochratoxin A in paired kidney and meat samples from healthy Danish slaughter pigs. Food Addit. Contam. 19, 562–567.
Jørgensen, K., 1998. Survey of pork, poultry, coffee, beer and pulses for ochratoxin A. Food Addit. Contam. 15, 550–554.
Logrieco, A., Bottalico, A., Mulé, G., Moretti, A., Perrone, G., 2003. Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. Eur. J. Plant Pathol. 109, 645–667.
Maaroufi, K., Achour, A., Hamammi, M., El May, M., Betbeder, A.M., Ellouz, F., Creppy, E.E., Bacha, H., 1995. Ochratoxin A in human blood in relation to nephropathy in Tunisia. Hum. Exp. Toxicol. 14, 609–615.
Magnoli, C.E., Astoreca, A.L., Chiacchiera, S.M., Dalcero, A.M., 2007. Occurrence of ochratoxin A and ochratoxigenic mycoflora in corn and corn based foods and feeds in some South American countries. Mycopathologia 163, 249–260.
Manique, R., Pena, A., Lino, C.M., Moltó, J.C., Mañez, J., 2008. OchratoxinA in the morning and afternoon portions of urine from Coimbra and Valencia populations. Toxicon 51, 1281–1287.

Märtlbauer, E., Usleber, E., Dietrich, R., Schneider, E., 2009. Ochratoxin A in human blood serum—retrospective long-term data. Mycotoxicol. Res. 25, 175–186.

Minerva, 2010. Ambiente e Diversidade na Europa: uma viagem pelos principais Biomas, Available at: http://www.minerva.uevora.pt/eschola2002/medit.htm.

Miraglia, M., Brera, C., 2002. Assessment of Dietary Intake of Ochratoxin A by the Population of EU Member States [SCOOP report Task 3.2.7.]. EU, Brussels.

Müller, G., Burkert, B., Möller, U., Diller, R., Rohrmann, B., Rosner, H., Köhler, H., 2004. Ochratoxin A and some of its derivatives modulate radical formation of porcine blood monocytes and granulocytes. Toxicology 199, 251–259.

Muñoz, K., Blaszkewicz, M., Degen, G.H., 2010. Simultaneous analysis of ochratoxin A and its major metabolite ochratoxin alpha in plasma and urine for an advanced biomonitoring of the mycotoxin. J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 878, 2623–2629.

Ng, W., Mankotia, M., Pantazopoulos, P., Neil, R.J., Scott, P.M., Lau, B.P., 2009. Survey of dry pasta for ochratoxin A in Canada. J. Food Protect. 72, 890–893.

Nikolov, I.G., Chernozemsky, I.N., Petkova-Bocharova, T., Vrabcheva, T., Stojchev, I., Bankov, B., Castegnaro, M., Pfohl-Leszkowicz, A., Dragacci, S., Day, N., Gill, C., Useleber, E., Dietrich, R., Martlbauer, E., 2002. Review of the cooperative studies of the national oncological centre in Bulgaria on Balkan endemic nephropathy and associated urinary tract tumours (1991–2001). Facta Univ. Ser. Med. Biol. 9, 119–122.

Ostry, V., Ruprich, J., Skarkova, J., 2002. Raisins, ochratoxin A and human health. Mycotoxicol. Res. 18 (S2), 178–182.

Pacin, A.M., Ciancio Bovier, E.V., Motta, E., Resnik, S.L., Villa, D., Olsen, M., 2008. Survey of Argentinean human plasma for ochratoxin A. Food Addit. Contam.—Part A 25, 635–641.

Palli, D., Miraglia, M., Saieva, C., Masala, G., Cava, E., Colatosti, M., Corsi, A.M., Russo, A., Brera, C., 1999. Serum levels of ochratoxin A in healthy adults in Tuscany: correlation with individual characteristics and between repeat measurements. Cancer Epidemiol. Biomark. Prevent. 8, 265–269.

Pena, A., Seifrtová, M., Lino, C.M., Silveira, M.I.N., Solich, P., 2006. Estimation of ochratoxin A in Portuguese population: new data on the occurrence in human urine by high performance liquid chromatography with fluorescence detection. Food Chem. Toxicol. 4, 1449–1454.

Peraica, M., Domijan, A.M., Matasin, M., Lucić, A., Radić, B., Delas, F., Horvat, M., Bosanac, I., Balija, M., Grgičević, D., 2001. Variations of ochratoxin A concentrations in the blood of healthy populations in some Croatian cities. Arch. Toxicol. 75, 410–414.

Petkova-Bocharova, T., Castegnaro, M., Pfohl-Leszkowicz, A., Garren, L., Grosso, F., Nikolov, I., Vrabcheva, T., Dragacci, S., Chernozemsky, I.N., 2003. Analysis of ochratoxin A in serum and urine of inhabitants from an area with Balkan endemic nephropathy: a one month follow up study. Facta Univ. Ser. Med. Biol. 10, 62–68.

Pfohl-Leszkowicz, A., Tozlovanu, M., Manderville, R., Peraica, M., Castegnaro, M., Stefanovic, V., 2007. New molecular and field evidences for the implication of mycotoxins but not aristolochic acid in human nephropathy and urinary tract tumor. Mol. Nutr. Food Res. 51, 1131–1146.

Rubert, J., Soriano, J.M., Mañes, J., Soler, C., 2011. Rapid mycotoxin analysis in human urine: a pilot study. Food Chem. Toxicol. 49, 2299–2304.

Sabolić, I., Asif, A.R., Budach, W.E., Wanke, C., Bahn, A., Burckhardt, G., 2007. Gender differences in kidney function. Pflugers. Arch. Eur. J. Physiol. 455, 397–429.

Schilter, B., Marin-Kuan, M., Delatour, T., Nestler, S., Mantle, P., Cavin, C., 2005. Ochratoxin A: potential epigenetic mechanisms of toxicity and carcinogenicity. Food Addit. Contam. 22 (S1), 88–93.

Shephard, G.S., 2008. Risk assessment of aflatoxins in food in Africa. Food Addit. Contam.—Part A 25, 1246–1256.

Soubra, L., Sarkis, D., Hilan, C., Verger, P., 2009. Occurrence of total aflatoxins, ochratoxin A and deoxynivalenol in foodstuffs available on the Lebanese market and their impact on dietary exposure of children and teenagers in Beirut. Food Addit. Contam.—Part A 26, 189–200.

Stander, M.A., Nieuwoudt, T.W., Steyn, P.S., Shephard, G.S., Creppy, E.E., Sewram, V., 2001. Toxicokinetics of ochratoxin A in vervet monkeys (*Cercopithecus aethiops*). Arch. Toxicol. 75, 262–269.

Stoev, S.D., 2010. Studies on carcinogenic and toxic effects of ochratoxin A in chicks. Toxins 2, 649–664.

Tafuri, A., Ferracane, R., Ritieni, A., 2004. Ochratoxin A in Italian marketed cocoa products. Food Chem. 88, 487–494.

Van der Merwe, K.J., Steyn, P.S., Fourie, L., Scott, D.B., Theron, J.J., 1965. Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. Nature 205, 1112–1113.

Vatinno, R., Aresta, A., Zambonin, C.G., Palmisano, F., 2008. Determination of ochratoxin A in green coffee beans by solid-phase microextraction and liquid chromatography with fluorescence detection. J. Chromatogr. A 1187, 145–150.

Visconti, A., Pascale, M., Centonze, G., 2000. Determination of ochratoxin A in domestic and imported beers in Italy by immunoaffinity clean-up and liquid chromatography. J. Chromatogr. A 888, 321–326.

Walker, R., Larsen, J.C., 2005. Ochratoxin A: previous risk assessments and issues arising. Food Addit. Contam.—Part A 22, 6–9.

Wangikar, P.B., Dwivedi, P., Sinha, N., Sharma, A.K., Telang, A.G., 2005. Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B(1) with special reference to microscopic effects. Toxicology 215, 37–47.

Wanigasuriya, K.P., Peiris, H., Ileperuma, N., Peiris-John, R.J., Wickremasinghe, R., 2008. Could ochratoxin A in food commodities be the cause of chronic kidney disease in Sri Lanka? Trans. R. Soc. Trop. Med. Hyg. 102, 726–728.

WHO—World Health Organization, 1995. Physical Status: The Use and Interpretation of Anthropometry. Report of a WHO Expert Committee [WHO Technical Report Series 854]. World Health Organization, Geneva.

Zimmerli, B., Dick, R., 1995. Determination of ochratoxin A at the ppt level in human blood, serum, milk and some foodstuffs by high-performance liquid chromatography with enhanced fluorescence detection and immunoaffinity column cleanup methodology and Swiss data. J. Chromatogr. B 666, 85–99.

Zinedine, A., Blesa, J., Mahnine, N., El Abidi, A., Montesano, D., Mañes, J., 2010. Pressurized liquid extraction coupled to liquid chromatography for the analysis of ochratoxin A in breakfast and infants cereals from Morocco. Food Control 21, 132–135.